Proximal tubular cell electrolytes during volume expansion in the rat

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1. Proximal tubular intracellular elements were measured by electron microprobe X-ray analysis (a) in rats volume-expanded with albumin–saline in which peritubular oncotic pressure remained normal and (b) in rats in which the renal artery was snared before volume expansion (the early snare model). Glomerular filtration rate and urine Na^+ excretion were measured in addition to intracellular Rb^+ following a 30 s infusion of RbCl as a marker for K^+ transport.

2. In albumin–saline volume-expanded rats, intracellular levels of Na^+ ($[Na^+]_i$) at $21{\cdot}5 \pm 0{\cdot}6$ mmol (kg wet wt)$^{-1}$, Cl^- ($[Cl^-]_i$) at $18{\cdot}0 \pm 0{\cdot}4$ mmol (kg wet wt)$^{-1}$ and Rb^+ ($[Rb^+]_i$) at $9{\cdot}4 \pm 0{\cdot}4$ mmol (kg wet wt)$^{-1}$ were significantly higher ($P < 0{\cdot}0001$) than the levels in non-expanded rats ($[Na^+]_i$, $[Cl^-]_i$ and $[Rb^+]_i$ at $17{\cdot}7 \pm 0{\cdot}4$, $14{\cdot}6 \pm 0{\cdot}3$ and $4{\cdot}7 \pm 0{\cdot}4$ mmol (kg wet wt)$^{-1}$, respectively; means ± S.E.M.). The data are consistent with Na^+ pump inhibition in the proximal tubule, although this cannot be directly derived from intracellular element measurements.

3. In an early snare model of volume expansion, $[Na^+]_i$, intracellular K^+ ($[K^+]_i$) and $[Rb^+]_i$ remained unchanged ($16{\cdot}1 \pm 0{\cdot}4$, $131{\cdot}0 \pm 2{\cdot}0$ and $5{\cdot}2 \pm 0{\cdot}3$ mmol (kg wet wt)$^{-1}$, respectively) compared to non-expanded snared kidneys ($15{\cdot}9 \pm 0{\cdot}6$, $131{\cdot}3 \pm 1{\cdot}8$ and $4{\cdot}8 \pm 0{\cdot}3$ mmol (kg wet wt)$^{-1}$, respectively). $[Cl^-]_i$ at $18{\cdot}3 \pm 0{\cdot}5$ mmol (kg wet wt)$^{-1}$ increased ($P < 0{\cdot}0008$) compared to controls at $15{\cdot}8 \pm 0{\cdot}5$ mmol (kg wet wt)$^{-1}$. Thus, in these rats, evidence for an inhibition of the Na^+ pump was no longer observed. This points to a major intrinsic mechanism within the kidney for mediating natriuresis, since circulating factors were identical to those in the unsnared kidney, where significant natriuresis occurred.

Electron microprobe X-ray (EMPX) analysis of proximal tubular cells from kidneys of rats undergoing sustained volume expansion induced by mannitol in saline has shown that intracellular Na^+ ($[Na^+]_i$) and intracellular Cl^- ($[Cl^-]_i$) are increased, consistent with inhibition of the Na^+ pump (György, Beck, Rick & Thurau, 1985).

In order to investigate the role of intrinsic mechanisms within the kidney, specifically peritubular oncotic pressure and renal perfusion pressure, in mediating the natriuresis of volume expansion, the following experiments were performed. Intracellular electrolyte concentrations were determined in rats volume-expanded with albumin–saline, which maintains normal peritubular oncotic pressure and therefore normal cell size, in contrast to previous experiments using mannitol, which increases peritubular oncotic pressure (György *et al.* 1985).

In an additional group of rats, volume expansion was induced with isotonic saline, but perfusion pressure was reduced in the experimental kidney. This manoeuvre is known to abolish natriuresis in that kidney (Fitzgibbons, Gennari, Garfinkel & Cortell, 1974) and abrogate the appearance of Na^+ transport inhibitor in proximal tubular fluid (Reddy, Cochineas & György, 1990).

Additional information regarding proximal tubular K^+ movement during volume expansion was gained by the technique of acute Rb^+ infusion (Beck, Dörge, Blümner, Giebisch & Thurau, 1988) followed by EMPX analysis of intracellular Rb^+.

METHODS

Studies were performed on male Wistar rats fed a standard rat cube diet (135 mg Na^+ per 100 g diet) and tap water.

Surgical preparation

Feed for the rats was withheld for 16 h (overnight) but they had free access to water. The rats were anaesthetized with I.P. Inactin (sodium 5-ethyl-5-(1-methylpropyl)-2-thiobarbiturate, 100 mg (kg body wt)$^{-1}$; Byk-Gulden, Konstanz, Germany) and placed on a thermostatically heated (37·5 °C) animal table. The trachea, left jugular vein and internal carotid artery were cannulated with polyethylene catheters. In control animals, saline (in mmol (kg wet wt)$^{-1}$ l^{-1}: NaCl, 150; KCl, 4) was infused through the jugular vein at 1·2 ml (100 g)$^{-1}$ h^{-1} using a Braun Perfusor. Blood sampling and continuous blood pressure monitoring (Statham-Gould Pressure Transducer) was performed through the carotid artery catheter. The rat was placed on its right side and the left kidney exposed through a flank incision. The kidney was freed of adherent fat and connective tissue and placed with the intact capsule in a metal cup as for micropuncturing. The left ureter was catheterized for collection of urine from the left kidney and the kidney was bathed in prewarmed paraffin oil. In rats in which perfusion pressure to the left kidney was reduced, additional catheters were placed into the left femoral artery and the urinary bladder. The urethra was tied off to ensure complete collection of urine from the right kidney. In these rats, the aorta in between the renal arteries was mobilized and a 3/0 silk suture (40 cm long) was placed around it. The ends of the suture were pulled through an 11 cm long polyethylene tube (1·2 mm o.d., 0·8 mm i.d.) for subsequent aortic constriction.

Clearance protocols

After completion of surgery, a priming dose of 53·28 kBq of [carboxyl-^{14}C]inulin (NEN, Boston, MA, USA) in 0·1 ml saline (100 g body wt)$^{-1}$ was rapidly injected through the jugular vein, followed by continuous infusion at 49·95 kBq (100 g)$^{-1}$ h^{-1} in 1·2 and 8 ml saline during non-expanded and volume-expanded periods, respectively. After a 30 min equilibration period, urine was collected under paraffin oil into preweighed tubes. Urine was collected for two 30 min periods during the non-expanded period (control) and for two 20 min periods during the volume expansion phase (experimental) except in time control experiments where no volume expansion was induced. At the mid-point of each urine collection period, 75 μl of blood was collected into microhaematocrit tubes. The radioactivity in 5 μl samples of plasma and urine were counted in a Rack-Beta 11 (LKB) liquid scintillation counter with automatic quench correction, which allowed measurement of GFR (glomerular filtration rate). Na^+ and K^+ in urine and plasma were measured with a flame photometer (IL 943). Urine and plasma osmolality were determined by vapour pressure osmometry (Wescor 5100C). At the end of clearance studies, a blood sample was obtained for plasma albumin determination using Bromocresol Green reagent (Technicon, Sydney, Australia) (Doumas, Watson & Biggs, 1971).

Experimental protocols

Group 1 (albumin–saline volume expansion). Five rats (252–304 g) were volume-expanded with albumin–saline after two control clearance periods. A bolus dose of albumin (40 mg (100 g)$^{-1}$ as 20% solution in saline) was infused into the jugular vein, followed by a maintenance dose of 30 mg in saline at 8 ml (100 g)$^{-1}$ h^{-1}. In preliminary experiments, these doses of albumin infusion were found to maintain the plasma albumin at control levels following volume expansion with saline. After the urine osmolality had decreased to 500 mosmol (kg H_2O)$^{-1}$ or less (45–60 min after volume expansion), two more clearance collections (20 min each) were made, before the kidney was prepared for excision and snap freezing for subsequent EMPX analysis (see below). Immediately prior to severing the renal pedicle and excision of the kidney, RbCl (BDH, Poole, Dorset, UK) at 0·33 ml (100 g)$^{-1}$ as a 150 mM isotonic solution was administered into the jugular vein over 30 s, as previously described (Beck *et al.* 1988; Beck, Dörge, Giebisch & Thurau, 1990).

Group 2. Four rats (260–289 g) were prepared as time controls for rats in experimental group 1. These rats received saline at 1·2 ml (100 g)$^{-1}$ h^{-1}, which maintains normal serum albumin. RbCl was administered as in group 1 and the kidneys were then prepared for EMPX analysis.

Group 3 (saline volume expansion with reduced renal perfusion pressure). In four rats (198–259 g), perfusion pressure to the left kidney was reduced to about 70 mmHg (monitored in the femoral artery) by tightening the snare around the aorta; volume expansion was begun at 8 ml saline (100 g)$^{-1}$ h^{-1} after control clearance periods. After the urine osmolality of the right (unsnared) kidney had decreased to 500 mosmol (kg H_2O)$^{-1}$ or less, two 20 min clearance periods were made using collections from both kidneys. RbCl was administered before the left kidney was excised for snap freezing.

Group 4. Four rats (180–264 g) were maintained as time controls for group 3 rats. Perfusion pressure to the left kidney was again reduced to about 70 mmHg and rats received saline at 1·2 ml (100 g)$^{-1}$ h^{-1} throughout the experiment. RbCl was administered and kidneys prepared for EMPX analysis.

At the completion of the experiments, the rats were killed with excess Inactin.

Preparation of the kidney for EMPX analysis

At the completion of the clearance studies, the ureter with the catheter was cut off and the capsule of the left kidney gently removed. The pedicle was freed of attachments. The oil in the cup and the abdominal cavity was aspirated and the kidney rinsed with a prewarmed salt solution containing (in mmol (kg wet wt)$^{-1}$ l^{-1}): NaCl, 109; $NaHCO_3$, 35; $MgCl_2$, 0·8; KCl, 5; $CaCl_2$, 1·5; Na_2HPO_4, 1; and blotted dry with a lint-free tissue. After several rinses, the kidney was covered with a thin layer of 20% albumin solution, freshly prepared with fraction V albumin (Sigma, St Louis, MO, USA) in a salt solution containing (in mmol (kg wet wt)$^{-1}$ l^{-1}): NaCl, 71; KCl, 4; $NaHCO_3$, 35; to act as a peripheral standard for subsequent EMPX analysis. The osmolality of this solution was adjusted to match to within 1–2% of the individual rat's plasma osmolality obtained immediately before preparation of the kidney for EMPX analysis. Following this the renal hilar structures were clamped and severed with sharp scissors. The kidney was then instantaneously transferred to a beaker containing a liquid isopentane–propane mixture cooled to liquid nitrogen temperature in a Dewar flask. The quench-frozen kidney, with its adherent albumin standard layer, was then transferred under liquid nitrogen to the electron microscope unit for further processing. Small pieces of the kidney cortex were sectioned at −90 °C using a Reichert FC4

cryosystem with a specially adapted steel knife. The cryosections, about 0·6 μm thick, were freeze-dried overnight at less than 10^{-3} Pa and EMPX analysis performed using techniques previously published (Dörge, Rick, Gehring & Thurau, 1978; Beck *et al.* 1980).

Briefly, EMPX analyses of individual proximal tubule cells (nuclear region) and of the standard albumin layer were made with a probe current of 0·5 nA at 20 kV (180 s counting time) in a Philips 505 scanning electron microscope modified for imaging of thin sections. Cell nuclei were used for analysis since, as previously demonstrated (Dörge *et al.* 1978), no gradients for Na^+ and K^+ exist across the nuclear membrane, and nuclear measurements exclude the possibility of sample area contamination by extracellular fluid components in brush-border or basolateral infoldings. X-rays were collected by an EDAX energy-dispersive detector and PV9900 multichannel analyser (EDAX International, Prairie View, IL, USA).

Spectra were deconvoluted using a filtered least-squares procedure with calibration corrections (Boström & Nockolds, 1989). Using the independently determined electrolyte concentrations obtained for the albumin standard solution, it was then possible to determine simultaneously the wet weight concentrations in mmol (kg wet wt)$^{-1}$ of Na^+, K^+, Rb^+ and Cl^- in the tubular epithelial cells.

Statistical analysis

Results are expressed as means ± S.E.M. Statistical differences between means were determined by analysis of variance (2-factor nested ANOVA: SuperANOVA®, Abacus concepts, Inc., 1984 Bonita Ave., Berkeley, CA 94704, USA) to remove the effect of variation due to rats not being their own controls, and the effect of large numbers of observations in a small group of rats.

RESULTS

Proximal tubular cell element concentrations

Intracellular element concentrations shown in Table 1 demonstrate that volume expansion with albumin–saline produced a significant increase in $[Na^+]_i$ (21·5%) and $[Cl^-]_i$ (23·3%) over the time control rats. These increases were similar to those obtained in mannitol–saline-expanded rats (György *et al.* 1985). Frequency distribution histograms for $[Na^+]_i$ concentrations are shown in Fig. 1. As in these earlier studies, $[K^+]_i$ did not change significantly. $[Rb^+]_i$ doubled during volume expansion with albumin–saline compared to time controls.

Cell dry weight was significantly higher (by 24%) in albumin–saline rats (27·7 ± 0·4%) compared to controls (22·2 ± 0·4%), indicating a decrease in cell volume. To confirm that cell volume is decreased during volume expansion irrespective of plasma or extracellular fluid osmotic or oncotic pressure, in a separate group of rats ($n = 5$) sustained volume expansion was induced with saline alone. Dry weight was increased from 19·3 ± 0·3% in control rats ($n = 3$) to 22·0 ± 0·3% ($P \geqslant 0{\cdot}0001$) in saline volume-expanded rats. Both $[Na^+]_i$ and $[Cl^-]_i$ were increased in these rats by 14·1% ($P = 0{\cdot}0008$) and 50·5%

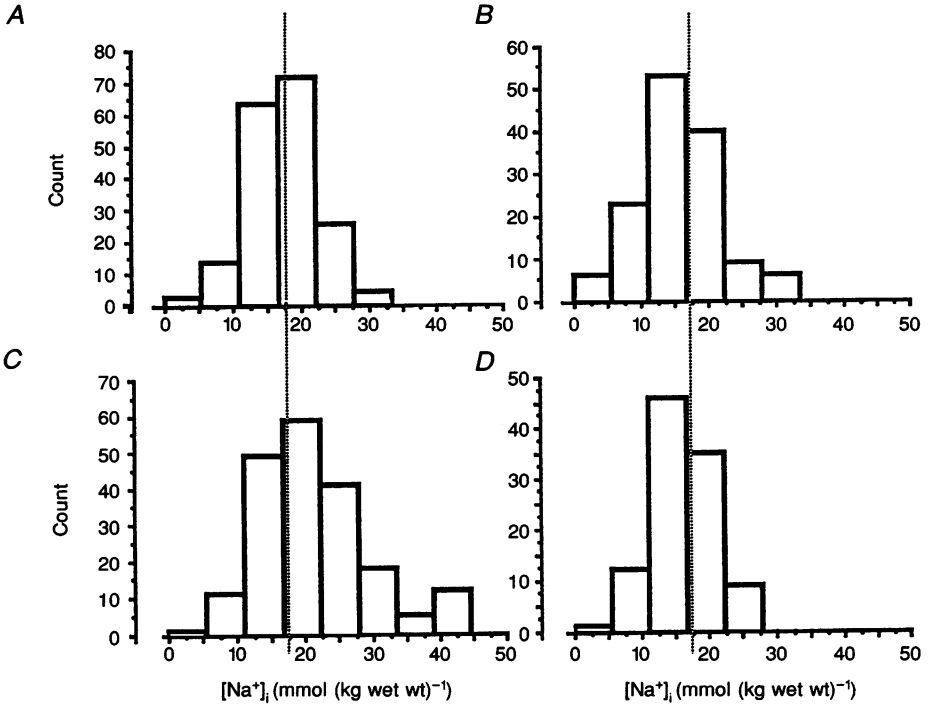

Figure 1. Frequency distribution for $[Na^+]_i$ concentrations in control and experimental proximal tubules of rat kidneys

A, time control for albumin–saline rats, non-volume-expanded; *B*, time control for snared rats, non-volume-expanded; *C*, albumin–saline volume-expanded rats; *D*, snared rats, saline volume-expanded. The dotted vertical line is placed at 17·5 mmol (kg wet wt)$^{-1}$ as a marker.

Table 1. Proximal tubular intracellular concentrations of N^+, Cl^-, K^+ and Rb^+ in kidneys in saline volume-expanded rats with a reduction in perfusion pressure (snare) to the experimental kidney and in albumin–saline volume-expanded rats

Experimental conditions	n	$[Na^+]_i$	$[Cl^-]_i$	$[K^+]_i$	$[Rb^+]_i$
Albumin–saline time control	180/4	17·7 ± 0·4	14·6 ± 0·3	123·4 ± 1·6	4·7 ± 0·4
Albumin–saline volume-expanded	196/5	21·5 ± 0·6	18·0 ± 0·4	124·1 ± 1·4	9·4 ± 0·4
P value		0·0001	0·0001	0·7	0·0001
Saline snare time control	137/4	15·9 ± 0·6	15·8 ± 0·5	131·0 ± 2·0	5·2 ± 0·3
Saline snare volume-expanded	105/4	16·1 ± 0·4	18·3 ± 0·5	131·3 ± 1·8	4·8 ± 0·3
P value		0·8	0·0008	0·9	0·4

Values are means ± S.E.M; n, number of estimations/number of animals; $[Na^+]_i$, $[Cl^-]_i$, $[K^+]_i$ and $[Rb^+]$: intracellular concentrations of Na^+, Cl^-, K^+ and Rb^+ in mmol (kg wet wt)$^{-1}$.

($P = 0·0001$), respectively, while $[K^+]_i$ remained unchanged. To confirm that changes in cell dry weight as determined by EMPX reflect true changes in cell size, in these groups of rats cell size was also measured by an independent method (in a blinded fashion) by image analysis of electron micrographs of proximal tubular cells obtained during EMPX analysis (TRACOR-Northern TN 8502). In the control animals, cell area was $866 \pm 44\ \mu m^2$ ($n = 46$) compared to $735 \pm 34\ \mu m^2$ ($n = 57$) ($P = 0·007$) in saline volume-expanded rats, representing an 18% decrease in cell size following volume expansion.

In the experiments where renal perfusion pressure was reduced before saline volume expansion, $[Na^+]_i$, $[Rb^+]_i$ and $[K^+]_i$ remained unchanged in the snared left kidney. However, $[Cl^-]_i$ remained significantly increased (15·8% higher than in time controls). Cell dry weights were comparable in the time control (21·1 ± 0·31%) and experimental snared kidneys (21·8 ± 4·1%).

Blood and urine data

Table 2 gives relevant blood and urine parameters measured in these rats. Based on haematocrit changes, there was significant volume expansion in both groups of rats from control to experimental periods. Plasma albumin concentrations were maintained in the albumin–saline group within the normal control range, as has previously been shown (Quinn & March, 1979).

Albumin–saline volume expansion produced significant increases in GFR ($P = 0·001$), urine flow and fractional excretion of Na^+ (FE_{Na}) ($P < 0·0001$) compared to the control period. In the snare experiments, the snared left kidney showed only mild diuresis and no increase in GFR and FE_{Na} during volume expansion, whereas the unsnared right kidney, acting as a time control for the left snared kidney, showed an increase in GFR ($P = 0·001$) and marked diuresis and natriuresis ($P = 0·0001$) following volume expansion.

Table 2. Blood and urinary parameters in time control and saline volume-expanded rats with a reduction in perfusion pressure (snare) to the experimental kidney, and in albumin–saline volume-expanded rats

Experimental conditions	n	MAP	GFR	V	FE_{Na}	$[Na^+]_p$	$[K^+]_p$	$[Alb]_p$	Ht
Albumin–saline time control									
Control period	4/8	121 ± 5	0·83 ± 0·14	1·6 ± 0·34	0·04 ± 0·01	144 ± 1·2	4·5 ± 0·7	—	51·6 ± 1·0
Exptl period	4/8	116 ± 6	1·04 ± 0·25	4·2 ± 2·10	0·15 ± 0·08	142 ± 3·3	4·3 ± 0·3	27·3 ± 3·8	51·1 ± 0·9
Albumin–saline volume-expanded									
Control period	5/10	113 ± 3	0·86 ± 0·08	1·2 ± 0·20	0·03 ± 0·01	143 ± 1·1	4·3 ± 0·2	—	48·9 ± 0·5
Exptl period	5/10	116 ± 3	1·66 ± 0·19	160 ± 31	7·00 ± 1·0	146 ± 2·2	4·1 ± 0·2	34·3 ± 2·7	40·4 ± 1·3
Saline snare time control									
Left kidney control period	4/8	110 ± 8	1·21 ± 0·14	2·9 ± 0·65	0·43 ± 0·11	147 ± 1·6	3·7 ± 0·4	—	49·3 ± 0·9
Right kidney control period	4/8	117 ± 7	1·24 ± 0·21	2·3 ± 0·17	0·39 ± 0·16				
Left kidney exptl period*	4/8	70 ± 2	0·64 ± 0·17	0·8 ± 0·17	0·07 ± 0·02	146 ± 1·3	4·0 ± 0·2	—	48·5 ± 0·9
Right kidney exptl period	4/8	115 ± 15	1·12 ± 0·20	4·9 ± 1·54	1·11 ± 0·75				
Saline snare volume-expanded									
Left kidney control period	4/8	126 ± 7	1·36 ± 0·15	2·8 ± 0·16	0·44 ± 0·13	142 ± 4·3	3·4 ± 0·5	—	49·0 ± 1·4
Right kidney control period	4/8	124 ± 5	1·27 ± 0·16	1·8 ± 0·17	0·24 ± 0·10				
Left kidney exptl period*	4/8	71 ± 3	1·55 ± 0·10	5·5 ± 0·90	0·73 ± 0·18	144 ± 4·2	3·6 ± 0·3	—	42·5 ± 0·9
Right kidney exptl period	4/8	131 ± 5	1·58 ± 0·22	122 ± 27	10·1 ± 1·0				

Values are means ± S.E.M; n, number of animals/collection periods; MAP, mean arterial pressure (mmHg); GFR, glomerular filtration rate (ml min^{-1} (g kidney wt)$^{-1}$); V, urine flow (μl min^{-1} per kidney); FE_{Na}, fractional excretion of Na^+ (%); $[Na^+]_p$, plasma Na^+ (mmol l^{-1}); $[K^+]_p$, plasma K^+ (mmol l^{-1}); $[Alb]_p$, plasma albumin (g l^{-1}); Ht, haematocrit (%); *snared kidney.

DISCUSSION

The current experiments demonstrate significant changes in proximal tubular intracellular electrolyte concentrations during albumin–saline volume expansion which are largely abrogated in the presence of reduced renal perfusion pressure. Specifically, following volume expansion with albumin–saline, $[Na^+]_i$ and $[Cl^-]_i$ were significantly increased, while $[K^+]_i$ remained unchanged. Furthermore, $[Rb^+]_i$ was markedly increased. In the volume-expanded animals with reduced renal perfusion pressure, both $[Na^+]_i$ and $[Rb^+]_i$ remained unchanged compared to controls while $[Cl^-]_i$ was again elevated. Since the increases observed in intracellular electrolyte concentrations in the present albumin–saline volume-expanded rats were also observed in mannitol–saline volume expansion (György *et al.* 1985) and saline volume expansion (see Results section), peritubular oncotic pressure is unlikely to be the factor responsible for the changes in cellular elemental concentrations.

The increased $[Na^+]_i$ during albumin–saline volume expansion is consistent with previous results and conclusions from this laboratory that under these conditions, Na^+ exit from the cell is reduced due to Na^+ pump inhibition (György *et al.* 1985). It is unlikely that the increase in $[Na^+]_i$ is a result of increased apical Na^+ entry. This is because net transtubular Na^+ and water transport have been previously shown to be reduced by 50% under similar conditions of volume expansion (György & Willis, 1983; Reddy *et al.* 1990; Reddy, György, Boström & Cochineas, 1991).

One other possibility which must be considered as the cause for changes in the intracellular electrolyte concentrations observed here and previously is that a reduction in proximal tubular cell size was responsible for these changes. Volume expansion, in the presence of normal renal perfusion pressure, causes a reduction in cell size independent of oncotic pressure (Bentzel, 1972; György *et al.* 1985; Maunsbach, Giebisch & Stanton, 1987; present data). However, the general increase in dry weight, reflecting reduced cell volume, is most likely to be related to cell compression due to increased interstitial hydrostatic pressure (Granger, Haas, Pawlowska & Knox, 1988) and luminal pressure (Bentzel, 1972) even in decapsulated kidneys (Khraibi & Knox, 1989). The type of fluid employed to produce volume expansion appears not to be relevant as the cell volume reduction was present with mannitol–saline (György *et al.* 1985), saline and albumin–saline models (given in this paper) with differing osmotic and oncotic properties. It is, however, unlikely that the increase in $[Na^+]_i$ in volume expansion is due to cell compression. This is because of the characteristics of the apical Na^+ entry–basal pump interplay (Hudson & Schultz, 1984; Schultz, 1992), where any rise in $[Na^+]_i$ in resting cells produced by physical compression would be expected to return to normal as a consequence of kinetic activation of the pump.

The rise in cell Na^+ during volume expansion is more likely to be due to the inhibition of Na^+ pump activity. Support for this view comes from previous studies where a proportion of the reduction in transepithelial transport seen in volume expansion could be attributed to a 'tubular factor'. This 'factor' appears in proximal tubular fluid during volume expansion and its action is transferable to non-volume-expanded kidneys (György & Willis, 1983; Reddy *et al.* 1990; Reddy *et al.* 1991). In experiments where perfusion pressure was reduced in the experimental kidney at the time of volume expansion, the effect of the tubular factor was no longer demonstrable (Reddy *et al.* 1990). This suggests that the change in intra-renal factors consequent upon volume expansion determine the natriuretic response and the increase in $[Na^+]_i$ (Lameire, Matthys, Mussche, Ringoir & Leusen, 1979; Granger *et al.* 1988). An increase in hydrostatic pressure *in vitro* has been reported to inhibit purified Na^+–K^+-ATPase from dog kidney (Chong, Fortes & Jamesen, 1985). It is not possible to distinguish from the present experiment to what proportion an increase in interstitial pressure, which we particularly tried to minimize by decapsulation, is responsible for the total Na^+ pump inhibition. Irrespective of circulating factors which may be present and contribute to the natriuresis in the opposite unsnared kidney, absence of natriuresis in the snared kidney demonstrates the primary role for mechanisms within the kidney in initiating sustained natriuresis.

The present experiments also demonstrate an elevation of $[Rb^+]_i$ in the animals volume-expanded with albumin–saline. A similar elevation of $[Rb^+]_i$ is inherent in the data reported from various earlier studies (Beck *et al.* 1988; Beck, Dörge, Giebisch & Thurau, 1988; Beck *et al.* 1990) with saline-only volume expansion. As Rb^+ reflects K^+ movement in cells, $[Rb^+]_i$ accumulation following the acute administration of RbCl has generally been considered to reflect activity of the basolateral Na^+–K^+-ATPase (Beck *et al.* 1988; Cheval & Doucet, 1990). This is likely to be the case when cell size does not differ between experimental groups. However, as passive movements of K^+ ions play a significant role in cell volume regulation, this interpretation may not apply in the current experiments where cell volume is altered. As the cells shrank but $[K^+]_i$ remained constant during volume expansion, it can be deduced that K^+ exited from the cell, presumably via an open K^+ (Rb^+) channel (Lang & Rehwald, 1992). Therefore, under these conditions, the rise observed in $[Rb^+]_i$ in the unsnared kidneys may be due to Rb^+ entering the cell through these channels down its large chemical gradient from outside to inside. It is not possible to say from the present experiments whether the affected channel is on the apical or basolateral side. During the 30 s of injection, the lumen of proximal tubules would certainly contain Rb^+ in addition to the plasma compartment.

$[Cl^-]_i$ was increased in both models of volume expansion studied. In the albumin–saline volume expansion model this is most likely to be the result of both increased cell content of Na^+ ions and a degree of drop in transepithelial resistance in proximal tubules found under these conditions (Seely, 1973; Sugo & György, 1990). The effect on transepithelial potential difference of reducing perfusion pressure to a kidney during volume expansion has, to our knowledge, not been investigated. Thus it can be deduced that the increase in $[Cl^-]_i$ found in the present experiments may reflect some transepithelial depolarization of the proximal tubular epithelium. This is not entirely unexpected since even though $[Na^+]_i$, $[Rb^+]_i$ and cell dry weight are unchanged and the effect of the tubular factor on transepithelial Na^+ and H_2O transport is abolished by the reduced perfusion pressure (Reddy *et al.* 1990), our previous work demonstrated that there was still a 20% reduction in Na^+ and H_2O transport in these kidneys (Reddy *et al.* 1990). This could be caused by circulating factors.

In summary, the present experiments support the view that the Na^+ pump is inhibited in proximal tubular cells during volume expansion, independent of peritubular oncotic pressure, and furthermore that intrinsic renal factors are significantly involved in the local mediation of this inhibitory effect.

REFERENCES

Beck, F., Bauer, R., Bauer, U., Mason, J., Dörge, A., Rick, R. & Thurau, K. (1980). Electron microscope analysis of intracellular elements in the rat kidney. *Kidney International* **17**, 756–763.

Beck, F.-X., Dörge, A., Blümner, E., Giebisch, G. & Thurau, K. (1988). Cell rubidium uptake: A method for studying functional heterogeneity in the nephron. *Kidney International* **33**, 642–651.

Beck, F.-X., Dörge, A., Giebisch, G. & Thurau, K. (1988). Renal excretion of rubidium and potassium: An electron microprobe and clearance study. *Kidney International* **34**, 455–162.

Beck, F.-X., Dörge, A., Giebisch, G. & Thurau, K. (1990). Effect of diuretics on cell potassium transport: An electron microprobe study. *Kidney International* **37**, 1423–1428.

Bentzel, C. J. (1972). Proximal tubule structure–function relationships during volume expansion in *Necturus*. *Kidney International* **2**, 324–335.

Boström, T. & Nockolds, C. E. (1989). Filtered least-squares fitting of EDS spectra with derivative references: problems and limitations imposed by counting statistics. In *Microbeam Analysis*, ed. Russell, P. D., pp. 233–235. San Francisco Press, San Francisco.

Cassola, A. C., Mollenhauer, M. & Frömter, E. (1983). The intracellular chloride activity of rat kidney proximal tubular cells. *Pflügers Archiv* **399**, 259–265.

Cheval, L. & Doucet, A. (1990). Measurement of Na-K-ATPase-mediated rubidium influx in single segments of rat nephron. *American Journal of Physiology* **259**, F111–121.

Chong, P. L. G., Fortes, P. A. G. & Jamesen, D. M. (1985). Mechanisms of inhibition of (Na, K)-ATPase by hydrostatic pressure studied with fluorescent probes. *Journal of Biological Chemistry* **260**, 14484–14490.

Dörge, A., Rick, R., Gehring, K. & Thurau, K. (1978). Preparation of freeze-dried cryosections for quantitative X-ray microanalysis of electrolytes in biological soft tissues. *Pflügers Archiv* **373**, 85–97.

Doumas, B. T., Watson, W. A. & Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta* **31**, 87–96.

Fitzgibbons, J. P., Gennari, F. J., Garfinkel, H. B. & Cortell, S. (1974). Dependence of saline-induced natriuresis upon exposure of the kidney to the physical effects of extracellular fluid volume expansion. *Journal of Clinical Investigation* **54**, 1428–1436.

Granger, J. P., Haas, J. A., Pawlowska, D. & Knox, F. G. (1988). Effect of direct increases in renal interstitial hydrostatic pressure on sodium excretion. *American Journal of Physiology* **254**, F527–532.

György, A. Z., Beck, F., Rick, R. & Thurau, K. (1985). Electron microprobe analysis of proximal tubule cellular Na, Cl and K element concentrations during acute mannitol–saline volume expansion in rats: evidence for inhibition of the Na pump. *Pflügers Archiv* **403**, 205–209.

György, A. Z. & Willis, W. (1983). Sodium transport inhibitor in proximal tubular urine during acute volume expansion. *Pflügers Archiv* **564**, 1–5.

Hudson, R. L. & Schultz, S. G. (1984). Effects of sodium coupled sugar transport on intracellular activities and sodium pump activity in *Necturus* small intestine. *Science* **224**, 1237–1239.

Khraibi, A. A. & Knox, F. G. (1989). Effect of acute renal decapsulation on renal interstitial hydrostatic pressure and natriuresis. *American Journal of Physiology* **256**, R44–48.

Lameire, N., Matthys, E., Mussche, M., Ringoir, S. & Leusen, I. (1979). Differences between dog and rat in the natriuretic response to extracellular volume expansion with immediate aortic constriction. *Journal of Laboratory and Clinical Medicine* **94**, 215–222.

Lang, F. & Rehwald, W. (1992). Potassium channels in renal epithelial transport regulation. *Physiological Reviews* **72**, 1–32.

Maunsbach, A. B., Giebisch, G. H. & Stanton, B. A. (1987). Effects of flow rate on proximal tubule ultrastructure. *American Journal of Physiology* **253**, F582–587.

Quinn, M. D. & Marsh, D. J. (1979). Peritubular capillary control of proximal tubule reabsorption in the rat. *American Journal of Physiology* **236**, F478–487.

Reddy, S., Cochineas, C. & György, A. Z. (1990). Differential Na fluxes with artificial and native fluids in clip models of volume expansion. *American Journal of Physiology* **259**, F480–484.

Reddy, S., György, A. Z., Boström, T. E. & Cochineas, C. (1991). Transfer of Na transport inhibition in proximal tubules from saline volume-expanded to nonexpanded rats. *American Journal of Physiology* **260**, F69–74.

Schultz, S. G. (1992). Membrane cross-talk in sodium-absorbing epithelial cells. In *The Kidney – Physiology and Pathophysiology*, ed. Seldin, D. W. & Giebisch, G., pp. 287–299. Raven Press, New York.

Seely, J. F. (1973). Effects of peritubular oncotic pressure on rat proximal tubule electrical resistance. *Kidney International* **4**, 28–35.

Sugo, E. & György, A. Z. (1990). Acute mannitol and saline volume expansion in the rat: effect on transepithelial potential difference in proximal tubules. *Clinical and Experimental Pharmacology and Physiology* **17**, 51–59.

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